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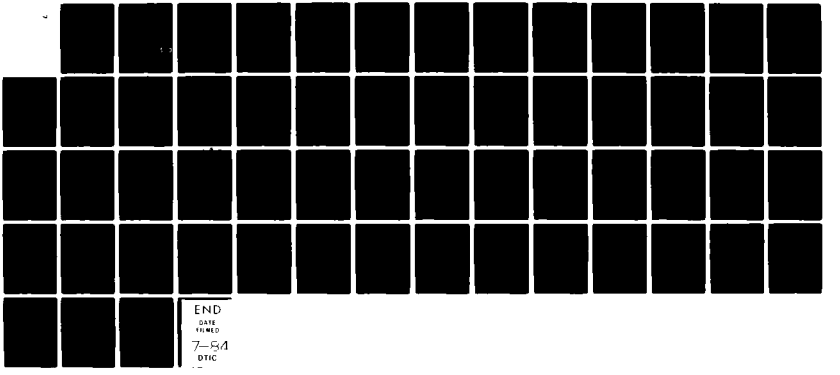

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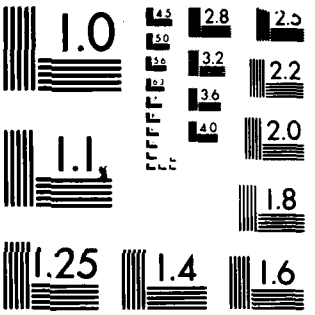

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MUTAGENIC SCREENING OF SIX CANDIDATE DYES FOR COLORED SMOKE MUNITIONS IN THE SALMONELLA REVERSION ASSAY

Final Report

Prepared by

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Dyes	Mutagenicity	
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Macrolex Violet B	Resiren Violet TR	

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Three red dyes, Amoplast Red PC, Macrolex Red 1069, and Oil Red G, and three violet dyes, Macrolex Violet B, Macrolex Violet 3R, and Resiren Violet TR were tested for mutagenic potential in the Salmonella reversion assay without and with metabolic activation by post-mitochondrial liver fractions induced with Aroclor or phenobarbital. The dyes which gave clearly positive mutagenic responses in the bacterial assay were Macrolex Red 1069, Amoplast Red PC, Resiren Violet TR, and Macrolex Violet B. The increase in revertants

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20. Abstract (continued)

was observed in those strains responsive to frameshift mutagens, TA1537, TA1538, and TA98. One dye was also active when tested with TA100 which can also detect frameshift mutagens.

Macrolex Red 1069 was active in TA1537 and TA1538 with and without activation and in TA98 only with activation.

Amoplast Red PC was active in TA1537, TA1538, TA98, and TA100 with and without activation.

Resiren Violet TR was active in TA1537, TA1538, and TA98 only with activation.

Macrolex Violet B gave a positive response only in TA1537 without activation.

FOREWORD

The *Salmonella* reversion assays performed by Oak Ridge National Laboratory were under the direction of Drs. J. Epler and T.K. Rao; and the assays conducted at the Health Effects Research Laboratory (HERL), US Environmental Protection Agency (USEPA) were supervised by Drs. J. Lewtas, Chief, and L. Claxton of the Genetic Bioassay Branch. Statistical analyses of the data were performed by HERL, USEPA using the test model and computer software developed by this group. The preparer wishes to express special appreciation to Dr. Claxton for evaluation of the statistical analyses and guidance in interpretation of their biologic significance.

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INTRODUCTION

Colored smoke munitions are used by the military as marking and signaling devices. These munitions contain organic dyes in a pyrotechnic formulation. Recent investigations[1] indicate that the dyes in current colored smoke munitions may be a health hazard to personnel manufacturing these munitions. An engineering study was performed by the US Army Chemical Research and Development Center to determine the feasibility of replacing the red dye in the M18 red smoke hand grenade and the red and violet dyes in the M18 violet grenade.[2] Two red dyes tested showed excellent potential of being technically acceptable in the M18 red smoke grenade. Two violet dyes emerged from the feasibility testing as candidates for further study.

A literature search for toxicologic data on these dyes was performed and very little information was obtained. The present studies were conducted to evaluate the potential of these dyes for producing bacterial mutagenesis. Six dyes were tested, three red and three violet, in the Salmonella typhimurium plate incorporation test. The three red dyes were retested in the same system by a second laboratory. The Salmonella procedure detects reverse mutations that occur in histidine-requiring strains developed by Dr. Bruce Ames.[3] Several different Salmonella strains are used to identify a variety of genetic mutational events. Each strain detects a specific type of mutation and incorporates other changes needed to increase its sensitivity. A mammalian metabolic activation system is added to the basic Salmonella assay to allow for some of the metabolites as well as the agent itself to be tested for mutagenicity.

MATERIALS AND METHODS

ORGANIC DYES

The red dyes tested in this study were

Oil Red G (Sudan R; Solvent Red 1; CI 12150)
Macrolex Red 1069
Amoplast Red PC (Red 10618)

The chemical structures of the last two dyes are unknown since the manufacturers regard this information as "commercial discrete." The Oil Red G is 1-(2-methoxyphenylazo)-2-naphthol.

The violet dyes tested were:

Resiren Violet TR (Disperse Violet 1; Solvent Violet II; CI 61100; 1,4-diaminoanthraquinone)
Macrolex Violet B (Solvent Violet 13; CI 60725; 1-toluidino-4-dihydroxyanthraquinone)
Macrolex Violet 3R (Solvent Violet 36)

The exact chemical structure of the latter dye is unpublished but it is an anthraquinone.

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The same samples of the dyes were provided to the two testing laboratories under the manufacturer's trade name without identification of the chemical structures. Chemical analyses for concentration of dye and identification of impurities were not done.

SALMONELLA PLATE INCORPORATION ASSAY

Bacterial Strains

Five histidine-requiring strains were used, three of which (TA1537, TA1538, and TA98) detect frameshift mutations. Chemicals that produce base-pair substitutions are detected in TA1535. Strain TA100 was developed from TA1535 by the addition of the R-factor plasmid. The R-plasmid causes a higher spontaneous mutation rate and can detect chemicals that normally yield frame-shift mutation. These strains carry an rfa mutation which produces a deficiency in bacterial cell wall lipopolysaccharides and increases the cell's permeability to large molecules, the UVR B mutation which decreases genetic repair, the R-factor plasmid in strains TA98 and TA100 which increases their sensitivity by participating in error-prone repair. The five strains differ in the number of spontaneous revertants per plate generally found. Compounds which are known mutagens for the different strains, with and without activation, were included in each assay. The retention of phenotypic characteristics were checked on a routine basis by examining for histidine auxotrophy (lack of growth on histidine deficient medium), deep rough character (sensitivity to crystal violet on a disk), UV-repair deficiency (sensitivity to UV light), and the presence of plasmid (resistance to ampicillin on disk).

Frozen permanent cultures containing fresh nutrient broth cultures with dimethyl sulfoxide were maintained at -80°C. A working source of these cultures was maintained on master plates. All strains were initially grown in nutrient broth at 37°C for 16 hours.

Preparation of Rat Liver S-9 Mix

Male rats weighing approximately 200 g were given a single intraperitoneal injection of Aroclor 1254 (Ar) in corn oil (200 mg/mL) at a dose of 500 mg/kg of body weight or 0.1 percent phenobarbital (Pb) in drinking water 5 days before they were killed. One day prior to termination the animals were taken off food but provided water ad libitum. The livers were asceptically removed and washed in sterile cold 0.15 M KCl. All subsequent steps were performed at 0° to 4°C with cold sterile solutions and sterile glassware. The livers were minced with scissors in 0.15 M KCl (3 mL/g wet weight liver) and homogenized with a Potter-Elvehjem homogenizer. The homogenate was centrifuged for 10 minutes at 9,000 x g, the supernatant (S-9) decanted and stored in convenient aliquots at -80°C.

The S-9 is mixed with a cofactor solution containing 8 µmol $MgCl_2$, 32 µmol KCl, 5 µmol glucose-6-phosphate and 4 µmol nicotinamide adenine dinucleotide in 100 µmol of sodium phosphate buffer, pH 7.4. The amount of S-9 used in the S-9 mix was between 0.05 and 0.1 mL S-9/mL cofactor solution.

Test Procedure

For revertant selection, minimal Vogel-Bonner medium E supplemented with 1.5 percent Difco bacto agar and 2 percent glucose was used for base agar layers. The top agar (0.6 percent Difco bacto agar, 0.5 percent NaCl) at 45°C was supplemented with minimum amounts of histidine and biotin, the bacterial broth culture ($1\text{-}2\times10^9$ viable cells per mL), and the test material dissolved in DMSO (supplied sterile, spectrophotometric grade). For tests without activation, 0.5 mL of buffer was added instead of the S-9 mix to the top agar. The plates were incubated in the dark at 37°C for 48 hours. The plates were examined for background growth and the number of colonies per plate was counted.

Statistical Analyses

Statistical tests were based upon the model by Stead et al.[4] This model assumes revertant colony formation at any dose follows a Poisson process, while the mean number of revertants per plate is a nonlinear function of up to four parameters. The resultant system of nonlinear equations is solved using a modified Gauss-Newton iterative scheme to obtain maximum likelihood estimates of the model parameters. Significance of the key parameters was tested by fitting reduced models and using likelihood ratio tests.

The determination of definite positives was based on the criteria outlined by Claxton et al:[5]

- The data must not vary significantly from a Poisson distribution ($p > 0.01$).
- The data must be acceptable by the test of adequacy ($p > 0.01$).
- The test for mutagenicity, the slope of the curve, must be significant ($p < 0.01$).
- At least a twofold increase must have occurred over spontaneous levels at one or more doses.
- A dose-response curve with some type of "regular curve" must have been seen.
- All controls must have given expected responses.
- Histidine cross-feeding and/or contamination must not have been shown to occur.

TESTING LABORATORIES

All six dyes were tested by ORNL, whereas only the three red dyes were tested by USEPA. There were differences in the bacterial mutagenesis protocols between the two laboratories. These variations in procedure were as follows:

Oak Ridge National Laboratory

Duplicate plates were usually used for each assay. The tests on each sample were generally repeated within 2 weeks following the initial evaluation to confirm the results. Results from three or more tests were compiled in evaluating the bioactivity of the test material. The tester strains were periodically checked with known chemical mutagens. The known carcinogens and the average responses of the five tester strains are listed in Table 1. The Sprague-Dawley rat strain was used to prepare the S-9 mix with both Aroclor and phenobarbital activation. A preincubation assay was also performed for all samples; the test sample was incubated with the bacterial cells and the activation mix (when appropriate) for 2 hours at 37°C before applying to the minimal media.

Genetic Toxicology Division, USEPA

The complete protocol for bacterial mutagenesis of the Genetic Toxicology Division, USEPA is outlined in Reference 5. This group uses the Charles River CD-1 rat for preparation of the S-9 mix with Aroclor activation only. Because the level of enzymatic activity can vary with each batch of S-9, various concentrations of the S-9 were tested within the bioassay against standard concentrations of three known mutagens; benzo[a]pyrene, 2-anthramine, and 7,12-dimethylbenzanthracene. In preparation of the liver homogenates, 0.25 M sucrose was the suspending medium and the stock solution also contained sucrose.

The minimum testing requirements were:

- A minimum of five doses at half-log intervals with the highest dose being highly toxic.
- Spontaneous and positive controls done at least in duplicate and providing the expected response.
- Positive controls (in duplicate) for the microsomal activation combination used are within normal ranges.
- At least two replicates per dose.
- At least one replicate experiment done with a narrower dose range.

The control mutagens for the five tester strains and the range of spontaneous revertants/plate are given in Table 2. The selection of positive controls was made from those listed; not all listed chemicals were used in each test.

RESULTS

The mean plate counts and standard deviations for each assay are given in Appendix A. The mutagenic indexes, defined as the average plate counts divided by the average spontaneous count for the same bacterial strain, are listed in Appendix B. The regression slopes, linear and nonlinear functions

as revertants per µg test material, are given in Appendix C. Dyes tested in the preincubation assay did not show greater activity than when tested in the plate incorporation assay and these data were not included in the analyses. The results from these preincubation assays are listed in the appendixes. The data from phenobarbital activation assays showed, in general, similar or lower activity than dyes incubated with Aroclor activated S-9 fractions.

The red dye, Macrolex Red 1069, was active in TA1537, TA1538, and TA98 (Table 3). Both testing laboratories reported increased revertants after Aroclor activation but only one laboratory reported positive results without S-9 activation in TA1537 and TA1538. The other laboratory used only two doses without activation, and the data could not be analyzed by the test model. This laboratory also reported positive results in TA1537, TA1538, and TA98 with phenobarbital activated microsomal fractions (Table B-2). Since the data did not give a good fit to the model, the results are not presented. Amoplast Red PC gave positive results in four tester strains, TA1537, TA1538, TA98, and TA100 (Table 4). The two testing laboratories reported similar results for the first three strains; the dye was active both with and without Aroclor activation. Only one laboratory reported activity in TA100, both with and without activation. The number of revertants per plate at similar doses per plate was markedly different between the two testing laboratories; revertant counts for TA1537 and TA1538 showed at least tenfold variations. The laboratory which reported positive results in TA100 also had the highest revertant counts in the three other strains. The inclusion of phenobarbital induced S-9 fractions gave a much lower mutagenic response in TA1537, TA1538, and TA98 than observed with Aroclor fractions (Table C-2). The red dye, Oil Red G, did not elicit an increase in revertants above the spontaneous count for each strain when tested in one laboratory. The other laboratory reported sporadic results with individual doses showing a greater than twofold increase over the spontaneous count but a dose-response relationship was not apparent (Table B-5).

The violet dye, Resiren Violet TR, was mutagenic for strains TA1537, TA1538, and TA98 with the S-9 liver fractions from both phenobarbital and Aroclor induced rats (Table 5). No increase in number of revertants per plate was apparent when the S-9 fraction was not activated. The mutagenic activity was apparent at low doses of dye per plate. Macrolex Violet B gave positive results only in TA1537 and only when nonactivated (Table 6). Macrolex Violet 3R did not give positive results under any assay conditions.

The comparisons of specific response activities for each of the mutagenic dyes across strains and activation systems are listed in Table 7. The specific response activity is the calculated dose in µg which gives a twofold increase over spontaneous revertants for a specific strain. The twofold increase was set at 50 revertants per plate for TA1537 and TA1538, 100 revertants for TA98, and 200 revertants for TA100. The doses were determined from each assay's regression slope calculated from the model curves; the slope functions were in the units revertants per µg. The higher value from the linear or nonlinear regression slope was used to more accurately represent the full range of concentration levels used in the tests.

A much greater dose of Macrolex Red 1069 was required to increase revertants per plate in TA1538 when not activated than when the activated microsomal fraction was incorporated into the test. There was little

difference in the dose required to give a doubling of spontaneous revertant number in TA1537 between tests using nonactivated and Aroclor activated fractions. Repetitive assays with this red dye in TA98 using activation showed a broad range of doses which would produce a twofold increase in spontaneous revertants, indicating marked day to day variation in the tests. The red dye, Amoplast Red PC, was also a more potent mutagen when the activation system was included in the test. The doses calculated from repeated tests with TA98 and TA100 showed less variability in specific response activity than noted for Macrolex Red 1069. In general, lower doses of Amoplast Red PC than Macrolex Red 1069 were required to produce a twofold increase in spontaneous revertants for TA1537, TA1538, and TA98. Resiren Violet TR was not a mutagen without S-9 activation. This dye was the only one which showed significant mutagenic activity when incubated with phenobarbital induced S-9 fractions. The phenobarbital microsomal fraction showed slightly greater enhancement of Resiren Violet TR activity than the Aroclor activation system in strains TA1538 and TA98 but not in strain TA1537.

DISCUSSION

The dyes which gave clearly positive mutagenic responses in the Salmonella reversion assay were Macrolex Red 1069, Amoplast Red PC, Resiren Violet TR and Macrolex Violet B. The dyes for which the assay results did not conform to the requirements for a positive response were Oil Red G and Macrolex Violet 3R. Significant increases in revertants were observed in those strains responsive to frameshift mutagens, TA1537, TA1538, and TA98. One red dye, Amoplast Red PC increased revertants in TA100 which can also detect frameshift mutagens. In general, those dyes showing a mutagenic response produced more revertants per plate when incubated with activated S-9 fractions than when the microsomal fraction had not been obtained from induced livers. The number of revertants was slightly greater in TA1538 and TA98 when Resiren Violet TR was incubated with a phenobarbital activated S-9 fraction rather than an Aroclor activated fraction. The violet dye, Macrolex Violet B however, did not give a positive response with the activated fractions, only with nonactivated fractions and in only one strain, TA1537. Additional studies are required with this dye to confirm the mutagenic activity detected in this bacterial assay. The slight increase in revertants elicited by Oil Red G was reported by only one laboratory, did not show a dose response, and was not considered a positive response. The increased number of revertants was only slightly above twofold the background number of revertants and was observed at high doses.

The specific response activities calculated from the data developed by the two laboratories did not show close numerical similarity. Even data from the same laboratory showed day to day variation in revertants per µg which was greater than would be expected. Part of this variability in numerical response may have been due to the insolubility of these dyes in aqueous media. Although DMSO was used as a suspending agent, some of the dye precipitated out during the incubation period. This would produce a variable concentration gradient across the plate, and not all bacteria would have been exposed to the same dosage level. This observation may also explain the differences between the laboratories in the strains which gave a positive response to the dye. The Salmonella reversion assay has approximately a 50 percent coefficient of variation for replicate experiments, even within the same laboratory (L. Claxton, personal communication). This level of variation

requires that individual assays be analyzed separately in the test model, and that only marked differences in numerical values between replications can be considered significant. The specific response activities should only be considered as guidelines in assessing the relative mutagenic potencies of test materials. Within the limitations of the bioassay, it appears that the dyes Amoplast Red PC and Resiren Violet TR were more potent mutagens in the Salmonella reversion test than Macrolex Red 1069 or Macrolex Violet B. The dyes tested in these studies are not as potent mutagens as the positive controls routinely used in this assay.

The frequent irregularity in the response of the strains to the dyes and the high levels of test materials required to obtain a mutagenic response suggests that the dyes are mixtures. More than one substance in the mixture may be mutagenic and contribute in a variable manner to the test materials' activity. Alternatively, the dyes may contain a mutagen which is present in low concentrations. The data from the Macrolex Red 1069 provide evidence for the presence of more than one active component since the with and without activation conditions gave different responses in different strains. If the mutagens are by-products of the dye synthesis process then different lots of the dye may give different results in mutagenic assays. These possibilities should be taken into consideration for future research on these dyes and other dyes considered for inclusion in colored smoke munitions.

TABLE 1. POSITIVE CONTROL MUTAGENS FOR THE FIVE TESTER STRAINS OF *SALMONELLA TYPHIMURIUM*, ORNL[a]

Tester Strain	DMSO	Revertants/Plate Without Activation	Mean Response	With Aroclor Activation	Mean Response	With Phenobarbital Activation	Mean Response
TA1535	15	Ethyl methane sulfonate	215				
		Sodium azide	557				
TA1537	11	8-Amino quinoline	62	Benzo[a]pyrene	171	8-Amino quinoline	428
		2-Nitrofluorene	103			2-Acetylamino-fluorene	46
TA1538	6	2-Nitrofluorene	615	Benzo[a]pyrene	94	2-Acetylamino-fluorene	1129
TA98	20	2-Nitrofluorene	586	Benzo[a]pyrene	245	8-Amino quinoline	57
						2-Acetylamino-fluorene	1367
TA100	103	Methyl methane sulfonate	504	Benzo[a]pyrene	639	8-Amino quinoline	263
		Sodium azide	748			2-Acetylamino-fluorene	1087
		2-Nitrofluorene	983				

a. ORNL - Oak Ridge National Laboratory.

TABLE 2. POSITIVE CONTROL MUTAGENS FOR THE FIVE TESTER STRAINS OF SALMONELLA TYPHIMURIUM, USEPA[a]

Tester Strain	Range of Spontaneous Revertants/Plate	Without Activation	Mean Response	With Aroclor Activation (+ S-9)	Mean Response
TA1535	5-50	N-Methyl-N'-nitrosoguanidine Sodium azide Methyl methane sulfonate	326	2-Anthramine	307
TA1537	2-25	9-Aminoacridine	1,202	2-Anthramine	630
TA1538	5-40	2-Nitrofluorene 4-Nitro-O-phenylenediamine	269	2-Anthramine	679
TA98	15-35	2-Nitrofluorene Hycanthone methane sulfonate	226	2-Anthramine	526
TA100	80-200	N-Methyl-N'-nitrosoguanidine Sodium azide Methyl methane sulfonate Nitrofurantoin	493	2-Anthramine	766

a. Genetic Bioassay Branch, US Environmental Protection Agency.

TABLE 3. MUTAGENICITY OF MACROLEX RED 1069[a]

Activation	µg/Plate	Mean Revertants Per Plate for Each Strain					
		TA1537		TA1538		TA98	
None	0	7		6			
	50	23		8			
	100	16		13			
	500	26		14			
	1,000	29		20			
	2,000	28		39			
	3,000	17		40			
Aroclor	0	7	(14)[b]	11	(14)	26	(32)
	50	28	(28)	20	(43)	45	(64)
	100	27		27		65	
	125		(42)		(64)		
	250		(57)		(105)		(142)
	500	30	(79)	77	(178)	142	(239)
	750		(81)		(208)		(252)
	1,000	57	(75)	133	(189)	208	(258)
	1,200			377			
	1,250		(167)		(333)		(474)
	2,000	113		302		463	
	2,500		(258)		(620)		(562)
	3,000	80				397	
	5,000		(396)		(949)	500	(865)
	7,500						(1,007)
	10,000		(398)				(1,035)

a. Only strains which had greater than twofold the number of spontaneous revertants and showed a dose response relationship are listed.

b. Data from ORNL in parentheses.

TABLE 4. MUTAGENICITY OF AMOPLAST RED PC[a]

Activation	µg/Plate	Mean Revertants Per Plate for Each Strain						
		TA1537		TA1538		TA98		TA100
None	0	13	(16)[b]	10	(8)	20	(33)	98
	10	21		41		120		180
	30	30		86		231		208
	50	32		86	(28)	241	(35)	221
	100	39		164		352		264
	125				(25)			
	150					457		291
	200					436		363
	250				(35)	1,222	(37)	328
	300	44		340		616		211
	500	33		356	(59)	614	(50)	379
	750				(63)		(66)	482
	1,000				(63)		(50)	642
	1,250		(44)		(112)		(88)	
	1,500							610
	2,500		(57)		(178)		(102)	555
	3,500							410
	5,000		(55)		(251)		(130)	
Aroclor	0	16	(13)	17	(15)	27	(42)	101
	10	28		82		357		227
	30	70		170		1,193		300
	50	100	(29)	328	(52)	1,514	(77)	382
	75				(63)		(73)	
	100	168		752	(56)	2,189	(82)	591
	120	196						
	125		(55)		(81)		(113)	
	150					2,002		710
	200	172				2,411		827
	250		(54)		(139)	2,005	(171)	791
	300			1,061		3,167		849
	500		(54)	1,137	(191)	2,966	(246)	792
	750		(54)		(209)		(272)	303
	1,000		(59)		(239)		(332)	210
	1,250		(107)		(361)		(423)	
	1,500							157
	2,500		(116)		(445)		(632)	84
	3,500							32
	5,000		(121)		(452)		(644)	

a. Only strains which had greater than twofold the number of spontaneous revertants and showed a dose response relationship are listed.

b. Data from ORNL in parentheses.

TABLE 5. MUTAGENICITY OF RESIREN VIOLET TR[a]

Activation	µg/Plate	Mean Revertants Per Plate for Each Strain TA1537	TA1538	TA98
Aroclor	0	14	15	42
	2.5		50	48
	5		63	90
	12.5	41	82	135
	25	64	127	171
	50	87	166	200
	125	90	143	209
	250	79	173	210
	500	88	188	235
	750	78		
	1,000	70		
	1,250	69		171
	2,500	51	76	144
	5,000	29	31	68
Phenobarbital	0	10	8	39
	1.25		53	87
	2.5		79	155
	5		115	170
	12.5	79	156	201
	25	90	171	203
	50	100	143	213
	125	105	158	239
	250	95	164	228
	500	83	159	208
	1,250	80		138
	2,500	50	73	94
	5,000	22	45	41

a. Only strains which had greater than twofold the number of spontaneous revertants and showed a dose response relationship are listed.

TABLE 6. MUTAGENICITY OF MACROLEX VIOLET B[a]

Activation	µg/Plate	Strain TA1537
None	0	12
	12.5	26
	25	33
	50	39
	125	47
	250	62
	500	49
	1,250	50
	2,500	75
	5,000	76

a. Only strains and doses which had greater than twofold the number of spontaneous revertants and showed a dose response relationship are listed.

TABLE 7. SPECIFIC RESPONSE ACTIVITY (µg)[a]

Compound	Activation[b]	Dose Calculated on Indicated Revertants/Plate: 50 TA1537	50 TA1538	100 TA98	200 TA100
Macrolex Red 1069	--	1,000	3,333		
	Ar	1,064	22	125 to 1,124	
Amoplast Red PC	--	126	31	25 to 47	223 to 615
	Ar	10	5	4 to 19	43 to 52
Resiren Violet TR	Ar	36	18	19	
	Pb	69	9	9	
Macrolex Violet B	--	301			

a. Specific response activity: Calculated dose needed to give approximately a twofold increase over spontaneous revertants for a specific strain. The doses were calculated from individual model curves and are either the linear or nonlinear model slope. The inclusion of ranges indicates more than one test was conducted with the strain.

b. --, No exogenous activation; Ar, S-9 from Aroclor 1254 induced rats; Pb, S-9 from phenobarbital induced rats.

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APPENDIX A

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM;
MEAN PLATE COUNTS AND STANDARD DEVIATION

Tables

TABLE A-1. MACROLEX RED 1069 IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM, WITHOUT ACTIVATION
USEPA

Compound	Dose, µg/Plate	Histidine Revertants Per Plate by Strain									
		TA1535	TA1537	TA1538	TA98				TA100		
DMSO	250	13[b] (3)	7 (2)	6 (2)	95[c] (22)	19 (3)	15 (1)	20 (1)	85 (6)	102 (13)	83 (9)
Positive[a] Control		228 (11)	1,118 (183)	211 (6)	588 (7)	188 (17)	218 (11)	211 (9)	448 (32)	611 (48	278 (36)
Red 1069	0.1					20 (6)	15 (1)		94 (4)	102 (0)	
	0.5					17 (1)	15 (4)		103 (1)	130 (4)	
	1					20 (1)	18 (1)		101 (17)	120 (27)	
	5					21 (4)	15 (4)		109 (1)	118 (11)	
	10				98 (0)	16 (0)	23 (8)		108 (11)	111 (1)	
	50	16 (4)	23 (2)	8 (2)	101 (1)	18 (2)	30 (3)	15 (2)	108 (6)	146 (6)	80 (16)
	100	20 (5)	16 (1)	13 (1)	95 99 (6) (1)	22 (4)	11 (1)	16 (3)	109 (22)	112 (9)	78 (9)
	500	16 (2)	26 (7)	14 (4)	90 99 (1) (4)	24	19 (1)	22 (2)	82 (3)	92 (3)	88 (9)
	1,000	21 (4)	29 (5)	20 (2)	88 102 (18) (1)	23	20 (3)	35 (2)	80 (1)	82 (3)	92 (7)
	2,000	16 (3)	28 (4)	39 (4)				42 (4)			92 (5)
	3,000	8 (2)	17 (5)	40 (8)				41 (8)			87 (18)
	5,000				111 101 (19) (3)	34	39 (1)		87 (1)	106 (1)	

a. Positive controls for strains: TA1535 and TA100, sodium azide, 1 µg/plate; TA1537, 9-aminoacridine, 100 µg/plate; TA1538 and TA98, 2-nitrofluorene, 3 µg/plate.
b. Mean of two to three replicates (standard deviation), except as noted.
c. Solvent was acetone.

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TABLE A-2. MACROLEX RED 1069 IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM, WITH S-9 ACTIVATION
USEPA

Compound	Dose, µg/Plate	Histidine Revertants Per Plate by Strain									
		TA1535	TA1537	TA1538	TA98				TA100		
DMSO	250	9[b] (4)	7 (2)	11 (3)	113[c] (1)	25 (5)	26 (1)	27 (5)	88 (4)	81 (5)	75 (8)
2-Anthramine	3[a]	293 (4)	861 (35)	479 (47)	899 (21)	448 (18)	471 (21)	277 (21)	670 (35)	807 (47)	337 (17)
	0.5										
Red 1069	0.1					30 (2)	27 (0)		85 (0)	114 (21)	
	0.5					24 (4)	33 (1)		96 (8)	136 (4)	
	1					25 (6)	33 (5)		101 (3)	122 (11)	
	5					33 (8)	33 (9)		108 (10)	135 (11)	
	10				119 (37)	45 (6)	33 (1)		112 (18)	138 (18)	
	50	13 (3)	28 (1)	20 (1)	120 (17)	55 (1)	41 (4)	40 (3)	101 (2)	126 (2)	73 (16)
	100	9 (4)	27 (1)	27 (4)	84 96 (21) (4)	79 (15)	54 (3)	61 (4)	90 (5)	103 (8)	81 (5)
	500	10 (5)	30 (4)	77 (14)	93 122 (13) (8)	132	166 (5)	130 (2)	90 (8)	104 (8)	87 (17)
	1,000	10 (2)	57 (10)	133 (15)	114 87 (7) (3)	191	214 (1)	219 (10)	83 (7)	86 (12)	92 (3)
	1,200			377 (38)							
	2,000	15 (3)	113 (13)	302 (29)				463 (32)			104 (8)
	3,000	14 (7)	80 (5)					397 (52)			104 (14)
	5,000				150 148 (69)(22)	471	528 (8)		116 (1)	121 (1)	

a. Doses for TA1535 and TA1538 were 3 µg/plate.
b. Mean of two to three replicates (standard deviation), except as noted.
c. Solvent was acetone.

TABLE A-3. AMOPLAST RED PC (RED 10618) IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM, WITHOUT ACTIVATION USEPA

Compound	Dose µg/Plate	Histidine Revertants Per Plate by Strain									
		TA1535	TA1537	TA1538	TA98			TA100			
DMSO	250	15[b] (2)	13 (1)	10 (4)	18 (1)	19 (3)	24 (3)	114 (1)	93 (0)	90 (1)	93 (19)
Positive[a] Control		348 (13)	1,191 (125)	220 (13)	275 (1)	188 (17)	223 (9)	721 (6)	493 (60)	448 (32)	495 (60)
Red 10618	10	17 (3)	21 (1)	41 (9)			120 (20)				180 (26)
	30	17 (4)	30 (6)	86 (11)			231 (6)				208 (15)
	50	21 (7)	32 (5)	86 (22)	203 (49)	237 (83)	284 (26)		207 (4)	239 (2)	217 (33)
	100	12 (3)	39 (6)	164 (33)	346 (43)	307 (4)	402 (21)		252 (32)	302 (8)	239 (17)
	150				513 (23)	400 (4)			235 (18)	347 (25)	
	200				459 (38)	413 (30)			317 (13)	408 (30)	
	250				628 (27)	1,816 (24)		398 (12)	278 (18)	307 (6)	
	300	18 (7)	44 (3)	340 (83)			616 (57)				211 (21)
	500	12 (2)	33 (4)	356 (18)	669 (10)	454 (11)	719 (49)	447 (1)	361 (21)	435 (26)	274 (47)
	750							482 (28)			
	1,000							642 (30)			
	1,500							610 (1)			
	2,500							555 (19)			
	3,500							410 (8)			

a. See Table A-1.
b. Mean of two to three replicates (standard deviation).

TABLE A-4. AMOPLAST RED PC (RED 10618) IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM, WITH S-9 ACTIVATION
USEPA

Compound	Dose µg/Plate	Histidine Revertants Per Plate by Strain									
		TA1535	TA1537	TA1538	TA98			TA100			
DMSO	250	11[b] (5)	16 (3)	17 (3)	23 (3)	25 (5)	34 (8)	121 (11)	90 (14)	88 (4)	103 (9)
2-Anthramine	3[a]	264 (57)	487 (47)	654 (120)	620 (30)	448 (18)	525 (23)	1,409 (158)	981 (122)	670 (35)	629 (77)
	0.5										
Red 10618	10	16 (2)	28 (1)	82 (17)			357 (8)				227 (3)
	30	15 (1)	70 (5)	170 (15)			1,193 (122)				300 (13)
	50	16 (6)	100 (14)	328 (14)	1,257 (122)	1,488 (68)	1,798 (39)		318 (47)	440 (11)	388 (23)
	100	19 (3)	168 (15)	752 (60)	2,076 (144)	2,228 (25)	2,263 (171)		504 (112)	639 (71)	630 (42)
	120		196 (16)								
	150				1,934 (32)	2,070 (118)			619 (52)	800 (23)	
	200		172 (22)		2,792 (4)	2,030 (45)			763 (115)	890 (146)	
	250				1,999 (88)	2,010 (89)		920 (87)	682 (37)	771 (50)	
	300	14 (1)		1,061 (186)			3,167 (37)				849 (43)
	500	16 (5)		1,137 (72)	3,016 (100)	2,841 (40)	3,042 (110)	541 (29)	987 (4)	871 (32)	868 (48)
	750							303 (42)			
	1,000							210 (35)			
	1,500							157 (48)			
	2,500							84 (11)			
	3,500							32 (5)			

a. Doses for TA1535 and TA1537 were 3 µg/plate.
b. Mean of two to three replicates (standard deviation).

TABLE A-5. OIL RED G IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM, WITHOUT ACTIVATION
USEPA

Compound	Dose ug/Plate	Histidine Revertants Per Plate by Strain															
		TA1535		TA1537		TA1538		TA98				TA100					
Acetone	100	18[b] (1)	17 (3)	5 (1)	10 (3)	10 (4)	18 (2)	17 (3)	24 (7)	16 (3)	22 (6)	67[c] (3)	95 (22)	121 (16)	114 (5)	86 (7)	113 (18)
Positive[a] Control		278 (58)	450 (83)	1,235 (124)	1,263 (138)	342 (12)	304 (6)	275 (1)	188 (17)	216 (11)	277 (14)	514 (106)	588 (7)	493 (60)	448 (32)	283 (17)	586 (13)
Oil Red G	5							28 (12)	22 (6)					112 (12)	120 (13)		
	10	15 (2)	18 (4)	7 (4)	13 (2)	10 (2)	11 (2)			20 (1)	18 (2)					75 (15)	89 (17)
	30	17 (3)	19 (3)	9 (1)	8 (2)	13 (3)	10 (2)			17 (5)	16 (5)					63 (2)	96 (6)
	50	16 (2)	19 (7)	12 (4)	10 (2)	8 (4)	12 (1)	18 (1)	13 (0)	18 (6)	20 (2)			101 (4)	117 (6)	65 (4)	102 (19)
	62.5											83 (4)					
	100	19 (4)	21 (4)	8 (1)	9 (3)	10 (2)	11 (2)	20 (1)	19 (3)	18 (2)	20 (2)		102 (13)	106 (13)	137 (26)	79 (13)	114 (10)
	125											67 (0)					
	250											82 (0)					
	300	17 (4)	21 (5)	9 (5)	12 (2)	14 (4)	16 (2)	17 (4)	23 (2)	18 (4)	20 (1)			113 (1)	97 (5)	76 (3)	104 (20)
	500	12 (0)	26 (4)	9 (5)	9 (2)	12 (2)	13 (2)	25 (6)	21 (7)	26 (9)	24 (5)	97 (2)	105 (4)	101 (5)	122 (2)	93 (7)	71 (15)
	1,000											93 (11)	91 (6)				
	1,500											92 (13)					
	5,000												116 (2)				

a. See Table A-1.
b. Mean of two to three replicates (standard deviation).
c. Negative control was DMSO, 250 ug/plate.

TABLE A-6. OIL RED G IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM, WITH S-9 ACTIVATION
USEPA

Compound	Dose ug/Plate	Histidine Revertants Per Plate by Strain															
		TA1535		TA1537		TA1538		TA98				TA100					
Acetone	100	15[b] (4)	16 (3)	8 (2)	10 (1)	21 (5)	26 (5)	30 (2)	29 (2)	28 (3)	35 (7)	86[c] (2)	113 (1)	138 (9)	101 (6)	92 (12)	94 (14)
2-Anthramine	3[a] 0.5	394 (19)	276 (22)	745 (44)	426 (20)	890 (19)	691 (29)	620 (30)	448 (18)	922 (85)	477 (28)	809 (37)	899 (21)	981 (122)	670 (35)	566 (9)	533 (50)
Oil Red G	5							44 (8)	32 (2)					159 (21)	204 (18)		
	10	11 (1)	11 (3)	10 (1)	12 (3)	16 (2)	19 (4)			25 (2)	33 (2)					153 (17)	145 (23)
	30	13 (2)	13 (1)	8 (2)	10 (1)	13 (2)	18 (2)			23 (4)	32 (3)					128 (18)	130 (16)
	50	9 (2)	12 (3)	20 (1)	13 (4)	16 (5)	21 (2)	35 (7)	29 (6)	31 (6)	33 (3)			153 (13)	179 (1)	130 (4)	131 (4)
	62.5											169 (26)					
	100	9 (1)	13 (1)	18 (2)	14 (3)	15 (6)	22 (5)	27 (2)	34 (1)	32 (5)	31 (3)		231 (18)	152 (15)	210 (7)	122 (13)	163 (28)
	125											147 (5)					
	250											140 (28)					
	300	9 (1)	10 (2)	10 (4)	12 (2)	16 (4)	19 (3)	38 (15)	35 (15)	26 (4)	30 (2)			158 (6)	177 (1)	142 (11)	132 (12)
	500	9 (2)	14 (4)	7 (1)	13 (2)	20 (2)	22 (1)	41 (6)	35 (6)	31 (3)	34 (6)	133 (13)	164 (23)	124 (4)	126 (16)	130 (10)	134 (17)
	1,000											153 (7)	171 (13)				
	1,500											150 (6)					
	5,000												136 (6)				

a. Doses for TA1535 and TA1537 were 3 ug/plate.
b. Mean of two to three replicates (standard deviation).
c. Negative control was DMSO, 250 ug/plate.

TABLE A-7. MACROLEX RED 1069 IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM AROCLOR ACTIVATION ORNL

Compound	µg/Plate	Activation[a]	Histidine Revertants Per Plate by Strain									
			TA1535	TA1537		TA1538		TA98		TA100		
DMSO	100	-		14 (9)		8 (2)		37 (19)		52 (10)		
		+	10[b] (4)	14 (6)		14 (4)		32 (17)		80 (10)		
Positive Control		-		118 (11)		237 (23)		355 (149)		205 (117)		
		+	91 (10)	289 (6)		237 (23)		355 (149)		205 (117)		
Red 1069	25	-		14 (3)								
	250	-		12 (2)		15 (4)		34 (16)		42 (13)		
	2,500					20 (9)		40 (19)		38		
	1.25	+	10 (2)									
	2.5	+	11 (6)									
	5	+	11 (6)									
	25	+	9 (3)	23 (5)	14 (4)	29 (10)	27 (0)	55 (19)		103 (30)		
	50	+	10 (2)	32 (9)	24 (5)	44 (12)	41 (6)	64 (16)		77 (6)		
	125	+		49 (6)	35 (0)	70 (17)	57					
	250	+	13 (5)	64 (17)	50 (8)	128 (44)	82 (8)	142 (35)		99 (14)		
	500	+	10 (8)	87 (28)	70 (8)	204 (75)	152 (9)	239 (109)		116 (17)		
	750	+		93 (8)	71 (0)	223 (54)	192	252 (82)				
	1,000	+		93 (28)	57 (0)	220 (55)	158	258 (42)				
	1,250	+		196 (60)	137 (0)	283	382 (61)	474 (157)				
	2,500	+	11 (6)	331 (72)	214 (17)	696 (113)	544 (143)	678 (145)	445 (44)	145 (32)		
	5,000	+	13 (4)	473 (114)	318 (22)	1,076 (247)	822 (68)	1,019 (125)	710 (110)	118 (50)		
	7,500	+						1,007 (113)				
	10,000	+		494 (135)	302			1,035 (137)				

a. - = No activation.
+ = With S-9 activation.
b. Mean of one to five replicates (standard deviation).

TABLE A-8. MACROLEX RED 1069 IN VITRO ASSAY WITH *SALMONELLA TYPHIMURIUM* PHENOBARBITAL ACTIVATION
ORNL

Compound	µg/Plate	Activation[a]	Histidine Revertants Per Plate by Strain TA1537	TA1538	TA98	TA100
DMSO	100	+	11[b] (3)	11 (1)	36 (15)	59 (6)
Positive Control		+	289 (6)	237 (23)	355 (149)	206 (118)
Red 1069	25	+	13 (4)	17 (3)	36 (11)	79 (15)
	50	+	20 (4)	19 (8)	38 (14)	67 (14)
	250	+	28 (4)	19 (2)	39 (9)	75 (18)
	500	+	26 (2)	36 (23)	50 (20)	67 (14)
	2,500	+	37 (11)	42 (17)	65 (20)	69 (6)
	5,000	+	38 (7)	78 (24)	93 (20)	61 (9)
	7,500	+		86 (37)	116 (9)	
	10,000	+		95 (8)	113 (29)	

a. - = No activation.
+ = With S-9 Activation
b. Mean of three to four replicates (standard deviation).

TABLE A-9. MACROLEX RED 1069 IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM PREINCUBATION
ORNL

Compound	µg/Plate	Activation[a]	Histidine Revertants Per Plate by Strain									
			Aroclor					Phenobarbital				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100	TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO	100	-	5[b] (5)	16 (9)	9 (1)	37 (9)	150 (22)					
		+	10 (2)	12 (10)	15 (5)	38 (21)	162 (56)	7 (3)	6 (1)	10 (8)	33 (22)	130 (17)
Positive Control		-	91 (10)	213 (85)	237 (23)	345 (163)	205 (117)					
		+	91 (10)	289 (6)	237 (23)	107 (15)	205 (117)	91 (10)	289 (6)	237 (23)	107 (15)	205 (117)
Red 1 69	25	-	8 (4)	19 (6)	14 (8)	36 (23)	107 (23)					
	2,5[illegible]	-	5 (3)	21 (6)	14 (8)	47 (19)	130 (36)					
	25	+	6 (3)	21 (3) 26	17 (4) 19	52 (15)	208 (63)	7 (4)	27 (10)	11 (2)	42 (19)	166 (59)
	5	+	8 (0)	16 (7) 24	18 (6) 17	33 (18)	128 (33)	5 (2)	15 (5)	10 (4)	44 (8)	147 (55)
	125	+			40 (14)	56 (7)						
	250	+	7 (1)	65 (22) 52	52 (26) 40	111 (54)	196 (75)	8 (2)	22 (7)	12 (2)	36 (21)	156 (63)
	500	+	7 (3)	56 (23) 43	94 (31)	147 (26)	163 (80)	5 (2)	11 (7)	10 (4)	26 (13)	130 (49)
	750	+		67 (29)	196 (52)	141 (51)						
	1,000	+		156 (23)	145 (9)	199 (75)						
	1,25[illegible]	+		278 (48)	321 (159)	400 (230)				13 (9)		
	2,5[illegible]	+	15 (4)	329 (134) 476	636 (175)	604 (301)	307 (105)	8 (6)	32 (11)	32 (5)	58 (22)	160 (50)
	3,5[illegible]	+			419 (268)					26 (13)		
	5,000	+	10 (5)	266 (93)	780 (515)	539 (198) 263	189 (65)	5 (3)	29 (9)	32 (19)	61 (12)	145 (40)
	7,500	+		253 (48)						20 (3)		
	10,000	+		129 (27)						23 (6)		

a. - = No activation.
+ = With S-9 activation.
b. Mean of two to three replicates (standard deviation), except where noted.

TABLE A-10. AMOPLAST RED PC (RED 10618) IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM
AROCLOR ACTIVATION
ORNL

Compound	µg/Plate	Histidine Revertants Per Plate by Strain									
		Without Activation					With Activation				
		TA 1535	TA 1537	TA 1538	TA 98	TA 100	TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO	100	9[a] (3)	16 (10)	8 (3)	33 (15)	64 (6)	8 (2)	13 (4)	15 (5)	42 (5)	89 (13)
Positive Control		90 (8)	213 (85)	237 (23)	355 (149)	205 (117)	91 (10)	118 (11)	237 (23)	355 (149)	205 (117)
Red 10618	12.5							18 (4)	23 (7)	59 (6)	
	25			14 (5)	42 (23)		7 (6)	22 (5)	29 (6)	56 (23)	109 (9)
	50			28 (3)	35 (15)		11 (7)	29 (4)	52 (26)	77 (13)	102 (6)
	75								63 (31)	73 (23)	
	100								56 (25)	82 (22)	
	125			25 (7)				55 (16)	81 (21)	113 (7)	
	250	3 (1)	19 (6) 15 (8)	35 (9)	37 (18)	19 (16)	9 (3)	54 (21)	139 (41)	171 (59)	116 (45)
	500		19 (12)	59 (8)	50 (21)		14 (4)	54 (14)	191 (60)	246 (55)	133 (48)
	750		20 (8)	63 (7)	66 (19)			54 (16)	209 (91)	272 (23)	
	1,000		15 (5)	63 (19)	50 (20)			59 (11)	239 (93)	332 (40)	
	1,250		44 (12)	112 (16)	88 (39)			107 (44)	361 (63)	423 (90)	
	2,500	4 (1)	66 (7) 47 (7)	178 (25)	102 (38)	8 (13)	13 (5)	116 (46)	445 (74)	632 (161)	130 (66)
	5,000		55 (8)	251 (62)	130 (64)		11 (2)	121 (27)	452 (80)	644 (281)	184 (28)
	7,500		47 (20)								
	10,000		44 (15)								

a. Mean of three to five replicates (standard deviation).

TABLE A-11. AMOPLAST RED PC (RED 10618) IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM PHENOBARBITAL ACTIVATION
ORNL

Compound	µg/Plate	Histidine Revertants Per Plate by Strain				
		TA1535	TA1537	TA1538	TA98	TA100
DMSO	100	10[a] (3)	10 (4)	8 (3)	39 (8)	70 (8)
Positive Control		90 (8)	118 (11)	237 (23)	355 (149)	205 (117)
Red 10618	12.5		24 (5)		51 (7)	
	25	10 (5)	28 (8)	24 (7)	63 (9)	74 (6)
	50	9 (3)	30 (5)	35 (6)	75 (5)	80 (7)
	125		35 (7)	47 (5)	83 (21)	
	250	11 (3)	42 (9)	69 (8)	100 (40)	83 (19)
	500	10 (4)	35 (5)	85 (23)	137 (33)	84 (16)
	750			93 (42)	173 (23)	
	1,000			101 (27)	173 (33)	
	1,250		85 (17)	183 (69)	240 (59)	
	2,500	9 (5)	100 (18)	253 (41)	367 (29)	94 (18)
	5,000	9 (1)	79 (28)	307 (14)	416 (88)	98 (6)

a. Mean of two to three replicates (standard deviation).

TABLE A-12. AMOPLAST RED PC (RED 10618) IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM PREINCUBATION ORNL

Compound	µg/Plate	Histidine Revertants Per Plate													
		Without Activation				Aroclor Activation				Phenobarbital Activation					
		TA 1535	TA 1538		TA 100	TA 1535	TA 1538		TA 98	TA 100	TA 1535	TA 1538		TA 98	TA 100
DMSO	100	9[a] (1)	9 (6)		112 (37)	12 (4)	18 (6)		35 (10)	130 (34)	14 (5)	20 (15)		32 (5)	117 (17)
Positive Control		91 (10)	237 (23)		175 (74)	91 (10)	237 (23)		107 (15)	205 (117)	91 (10)	237 (23)		107 (15)	205 (117)
Red 10618	25		15 (12)			8 (1)	27 (12)	10 (0)	37 (15)	122 (37)	10 (3)	16 (9)	9 (1)	17 (3)	109 (25)
	50		18 (11)			9 (2)	27 (13)	14 (8)	32 (12)	98 (31)	17 (4)	19 (13)	12 (1)	26 (4)	96 (27)
	250	11 (2)	37 (19)	29	127 (51)	9 (1)	69 (15)	35 (22)	51 (12)	112 (26)	12 (2)	27 (15)	21 (3)	35 (14)	112 (49)
	500		37 (20)	13		10 (1)	70 (34)	27 (1)	51 (17)	88 (30)	19 (7)	45 (13)	16 (1)	33 (9)	86 (34)
	750		38 (26)	12			48 (18)	18	46 (15)			38 (13)	17	46 (16)	
	1,000		44 (31)	11			56 (37)	10	57 (24)			69 (30)	36	66 (23)	
	1,250		180 (50)	138			169 (73)	58	125 (40)			60 (21)	42	80 (24)	
	2,500	14 (8)	252 (91)	237 (40)	137 (71)	15 (3)	172 (64)	94 (26)	152 (60)	157 (48)	9 (3)	108 (41)	104	134 (42)	170 (70)
	3,750		319 (96)	179			179 (66)	133	106 (24)			129 (38)	97 (47)	100 (22)	
	5,000					10 (1)	206 (56)	118 (27)	158 (53)	121 (29)	12 (3)	182 (44)	110	239 (238)	169 (62)
	7,500		305 (104)	258			273 (88)	120				265 (40)	93 (6)		
	10,000		299 (151)	137			342 (134)	185				223 (86)	126		

a. Mean of one to five replicates (standard deviation).

TABLE A-13. OIL RED G IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM PLATE INCORPORATION ASSAY
ORNL

Compound	ug/Plate	Activation[a]	Histidine Revertants Per Plate by Strain				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO	100	--	11[b] (3)	14 (9)	8 (2)	37 (19)	52 (10)
		Ar	10 (4)	14 (5)	14 (4)	32 (17)	80 (10)
		Pb	11 (7)	11 (3)	11 (1)	36 (15)	59 (6)
Positive Control		--	91 (10)	118 (11)	237 (23)	355 (149)	206 (118)
		Ar	91 (10)	289 (66)	237 (23)	355 (149)	535 (117)
		Pb	91 (10)	289 (66)	237 (23)	355 (149)	205 (117)
[illegible]	[illegible]	--	[illegible] (3)				
	[illegible]	--	[illegible] (4)	[illegible] (14)	[illegible] (4)	[illegible] (39)	--[c]
	[illegible]	--	[illegible] (3)	[illegible]		[illegible] (19)	--[c]
	[illegible]				[illegible] (3)		
	[illegible]	Ar	[illegible] (6)	[illegible] (6)	[illegible] (3)	[illegible] (6)	[illegible] (13)
	[illegible]		[illegible] (5)	[illegible] (5)	[illegible] (11)	[illegible] (15)	[illegible] (23)
	[illegible]		[illegible] (8)	[illegible] (3)	[illegible] (2)	[illegible] (14)	[illegible] (5)
	[illegible]		[illegible] (6)	[illegible] (2)	[illegible] (2)	[illegible] (3)	[illegible] (17)
	[illegible]		[illegible] (3)	[illegible] (3)	[illegible] (5)	[illegible] (13)	[illegible] (8)
	[illegible]		[illegible] (13)	[illegible] (3)	[illegible] (7)	[illegible] (9)	[illegible] (3)
	[illegible]	Pb	[illegible] (2)	[illegible] (3)	[illegible] (2)	[illegible] (3)	[illegible] (22)
	[illegible]		[illegible] (3)	[illegible] (3)	[illegible] (3)	[illegible] (15)	[illegible] (25)
	[illegible]		[illegible] (4)	[illegible] (3)	[illegible] (3)	[illegible] (8)	[illegible] (15)
	[illegible]		[illegible] (7)	[illegible] (1)	[illegible] (1)	[illegible] (10)	[illegible] (6)
	[illegible]		[illegible] (7)	[illegible] (7)	[illegible] (4)	[illegible] (4)	[illegible] (21)

a. -- = No activation; Ar = Aroclor; Pb = Phenobarbital.
b. Mean of two to three replicates (standard deviation).
c. Toxic to bacteria.

TABLE A-14. OIL RED G IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM
PREINCUBATION ASSAY
ORNL

Compound	µg/Plate	Activation[a]	Histidine Revertants Per Plate by Strain				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO	100	—	5[b] (5)	16 (9)	11 (5)	37 (9)	150 (22)
		Ar	10 (2)	11 (9)	15 (5)	38 (21)	162 (56)
		Pb	7 (3)	6 (1)	10 (8)	34 (23)	130 (17)
Positive Control		—	91 (10)	213 (83)	237 (23)	345 (163)	205 (116)
		Ar	91 (10)	289 (6)	237 (23)	107 (15)	205 (117)
		Pb	91 (10)	289 (6)	237 (23)	107 (15)	205 (117)
Oil Red G	250	—	10 (6)	—[c]	5 (0)	29[c]	148 (6)
	2,500	—	7 (3)	—[c]	11 (0)	17[c]	86[c]
	25	Ar	9 (7)	11 (5)	13 (8)	40 (24)	184 (80)
	50		10 (2)	20 (4)	13 (3)	37 (17)	155 (78)
	250		11 (2)	13 (3)	11 (4)	35 (20)	174 (57)
	500		7 (3)	13 (2)	10 (3)	38 (8)	150 (43)
	2,500		8 (2)	12 (5)	16 (3)	33 (18)	170 (51)
	5,000		6 (4)	14 (6)	17 (3)	38 (8)	174 (65)
	25	Pb	6 (3)	9 (2)	8 (3)	28 (10)	127 (60)
	50		5 (1)	6 (2)	8 (3)	18 (6)	127 (38)
	250		12 (5)	6 (3)	8 (2)	27 (10)	90 (16)
	500		6 (5)	9 (3)	6 (2)	32 (18)	96 (23)
	2,500		8 (3)	8 (3)	11 (3)	25 (6)	147 (46)
	5,000		6 (1)	8 (4)	10 (4)	30 (12)	107 (23)

a. — = No activation; Ar = Aroclor; Pb = Phenobarbital.
b. Mean of three to five replicates (standard deviation).
c. Toxic to bacteria.

TABLE A-15. RESIREN VIOLET TR IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM
PLATE INCORPORATION ASSAY
ORNL

Compound	µ/Plate	Activation[a]	Histidine Revertants Per Plate by Strain									
			Aroclor Activation					Phenobarbital Activation				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100	TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO	100	-	9[b] (3)	14 (4)	8 (3)	34 (14)	64 (6)					
		+	8 (2)	14 (4)	15 (5)	42 (5)	89 (13)	10 (3)	10 (4)	8 (3)	39 (8)	70 (8)
Positive Control		-	90 (8)	213 (85)	237 (23)	355 (149)	205 (117)					
		+	91 (10)	118 (11)	237 (23)	355 (149)	205 (117)	90 (8)	118 (11)	237 (23)	355 (149)	205 (117)
Violet TR	250	-	7 (4)	39 (21)	15 (10)	30 (16)	15 (9)					
	2,500	-	6[c] (1)	25 (18)	16 (10)	22 (19)	4 (5)					
	1.25	+			26 (15)	51 (15)				53 (13)	87 (28)	
	2.5	+			30 (25)	48 (16)				79 (10)	155 (21)	
	5	+			63 (22)	90 (26)				115 (18)	170 (30)	
	12.5	+		41 (10)	82 (37)	135 (30)			79 (4)	156 (12)	201 (59)	
	25	+	11 (5)	64 (14)	127 (19)	171 (39)	82 (24)	8 (3)	90 (6)	171 (27)	203 (53)	76 (4)
	50	+	3 (1)	87 (23)	166 (38)	200 (64)	72 (18)	10 (6)	100 (3)	143 (26)	213 (48)	81 (3)
	125	+		90 (13)	143 (57)	209 (41)			105 (5)	158 (28)	239 (73)	
	250	+	7 (3)	79 (16)	173 (52)	210 (45)	89 (21)	11 (8)	95 (5)	164 (22)	228 (62)	88 (14)
	500	+	3 (2)	88 (14)	188 (53)	235 (22)	79 (18)	7 (3)	83 (6)	159 (18)	208 (42)	75 (6)
	750	+		78 (25)								
	1,000	+		70 (23)								
	1,250	+		69 (6)		171 (54)			80 (4)		138 (46)	
	2,500	+	3 (4)	51 (21)	76 (11)	144 (45)	45 (14)	7 (6)	50 (15)	73 (14)	94 (25)	40 (7)
	5,000	+	1 (2)	29 (17)	31 (7)	98 (45)	5 (2)	5 (1)	22 (10)	45 (7)	41 (24)	30 (25)

a. - = No activation; + = With S-9 activation.
b. Mean of three to five replicates (standard deviation).
c. Toxic to bacteria.

TABLE A-16. RESIREN VIOLET TR IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM
PREINCUBATION ASSAY
ORNL

Compound	µg/Plate	Histidine Revertants Per Plate by Strain											
		Without Activation				Aroclor Activation				Phenobarbital Activation			
		TA 1535	TA 1538	TA 98	TA 100	TA 1535	TA 1538	TA 98	TA 100	TA 1535	TA 1538	TA 98	TA 100
DMSO	100	9[a] (1)	9 (6)		112 (37)	12 (4)	18 (6)	35 (10)	130 (34)	14 (5)	20 (15)	32 (5)	117 (17)
Positive		91 (10)	237 (23)	355 (149)	205 (117)	91 (10)	237 (22)	107 (15)	205 (117)	91 (10)	237 (23)	107 (15)	205 (117)
	25			22 (10)		11 (1)	29 (9)	70 (18)	101 (22)	11 (5)	36 (11)	49 (8)	
	50			37 (20)		8 (2)	38 (14)	58 (14)	82 (36)	10 (3)	34 (5)	48 (12)	100 (23)
	125			47 (1)									
	250	9 (6)	22 (4)	46 (9)	117 (30)	6 (2)	50 (9)	68 (12)	105 (27)	9 (1)	31 (14)	52 (18)	80 (17)
	500			40 (2)		7 (2)	43 (4)	57 (18)	77 (33)	8 (3)	30 (7)	34 (13)	81 (12)
	750			33 (12)									
	1,000			12 (0)									
	1,250			37 (13)									
	2,500	5 (1)	18 (15)	41 (13)	48 (16)	10 (3)	32 (6)	47 (14)	75 (19)	7 (2)	17 (6)	32 (9)	46 (4)
	3,750			38 (14)									
	5,000			27 (8)		10 (3)	26 (3)	43 (21)	59 (24)	4 (2)	17 (6)	17 (3)	76 (1)

a. Mean of two to five replicates (standard deviation).

TABLE A-17. MACROLEX VIOLET B IN VITRO ASSAY WITH *SALMONELLA TYPHIMURIUM* PLATE INCORPORATION ASSAY
ORNL

Compound	µg/Plate	Activation[a]	Histidine Revertants Per Plate by Strain				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO	100	-	11[b] (3)	12 (9)	8 (2)	37 (19)	52 (10)
		Ar	10 (4)	14 (5)	14 (4)	32 (17)	80 (10)
		Pb	11 (7)	11 (3)	11 (1)	36 (15)	59 (6)
Positive Control		-	91 (10)	118 (11)	237 (23)	355 (149)	205 (117)
		Ar	91 (10)	289 (6)	237 (23)	355 (149)	205 (117)
		Pb	91 (10)	289 (6)	237 (23)	355 (149)	205 (117)
Violet B	12.5	-		26 (11)			
	25	-	10 (2)	33 (13)			
	50	-		39 (17)			
	125	-		47 (8)			
	250	-	11[c] (5)	62 (27)	10 (3)	47 (20)	48[c] (8)
	500	-		49 (12)			
	1,250	-		50 (25)			
	2,500	-	10[c] (6)	75 (22)	11[c] (7)	41 (13)	48[c]
	5,000	-		76 (28)			
	25	Ar	11 (6)	12 (4)	20 (3)	38 (11)	77 (17)
	50		12 (5)	13 (3)	20 (7)	43 (9)	59 (8)
	250		11 (4)	16 (4)	24 (4)	44 (15)	71 (12)
	500		10 (6)	13 (4)	27 (6)	37 (4)	76 (17)
	2,500		11 (6)	25 (3)	27 (4)	46 (18)	72 (16)
	5,000		9 (7)	24 (3)	29 (2)	45 (11)	64 (9)
	25	Pb	7 (5)	11 (2)	11 (2)	31 (12)	54 (11)
	50		10 (6)	7 (2)	10 (4)	35 (14)	62 (15)
	250		10 (7)	10 (2)	10 (4)	32 (18)	64 (9)
	500		14 (1)	10 (3)	11 (4)	31 (6)	64 (15)
	2,500		12 (5)	15 (3)	16 (3)	36 (6)	67 (13)
	5,000		9 (3)	18 (3)	13 (6)	35 (12)	58 (13)

a. Ar = Aroclor; Pb = Phenobarbital.
b. Mean of two to three replicates (standard deviation).
c. Toxic to bacteria.

TABLE A-18. MACROLEX VIOLET B IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM PREINCUBATION ASSAY

Compound	µg/Plate	Activation[a]	Histidine Revertants Per Plate by Strain				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO	100	-	5[b] (5)	16 (9)	11 (5)	37 (9)	150 (22)
		Ar	10 (2)	11 (9)	15 (5)	32 (18)	162 (56)
		Pb	7 (3)	6 (1)	10 (8)	34 (23)	130 (17)
Positive Control		-	9 (10)	213 (85)	237 (23)	345 (163)	205 (117)
		Ar	91 (10)	289 (6)	237 (23)	107 (15)	205 (117)
		Pb	91 (10)	289 (6)	237 (23)	107 (15)	205 (117)
Violet B	25	-		16 (1)			
	50	-		18 (5)			
	125	-		29 (14)			
	250	-	9 (4)	45 (23)	8 (4)	15 (1)	144 (21)
	500	-		53 (26)			
	1,250	-		71 (37)			
	2,500	-	7 (1)	57 (16)	9 (2)	19 (4)	120 (5)
	5,000	-		61 (12)			
	7,000	-		67 (36)			
	10,000	-		99 (19)			
	25	Ar	12 (4)	11 (3)	15 (3)	45 (16)	140 (30)
	50		8 (4)	8 (2)	15 (5)	35 (12)	107 (41)
	250		8 (3)	7 (3)	13 (4)	47 (20)	117 (10)
	500		8 (1)	9 (4)	12 (1)	40 (17)	112 (25)
	2,500		7 (3)	18 (4)	21 (4)	38 (24)	162 (21)
	5,000		7 (2)	21 (10)	16 (3)	26 (4)	121 (54)
	25	Pb	13 (3)	9 (4)	12 (5)	36 (6)	115 (9)
	50		7 (2)	9 (4)	9 (5)	30 (20)	114 (21)
	250		10 (2)	10 (4)	13 (4)	29 (8)	146 (29)
	500		6 (2)	5 (1)	12 (5)	30 (11)	115 (61)
	2,500		8 (6)	16 (4)	15 (4)	35 (13)	104 (28)
	5,000		5 (3)	16 (3)	11 (2)	38 (20)	101 (9)

a. - = No activation; Ar = Aroclor; Pb = Phenobarbital.
b. Mean of two to five replicates (standard deviation).

TABLE A-19. MACROLEX VIOLET 3R IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM PLATE INCORPORATION ASSAY
ORNL

Compound	µg/Plate	Activation[a]	Histidine Revertants Per Plate by Strain				
			TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO	100	-	9[b] (3)	14 (8)	8 (3)	34 (14)	64 (6)
		Ar	8 (2)	13 (4)	15 (5)	42 (5)	89 (13)
		Pb	10 (3)	10 (4)	8 (3)	39 (8)	70 (8)
Positive Control		-	90 (8)	213 (85)	237 (23)	355 (149)	205 (117)
		Ar	91 (10)	119 (13)	237 (23)	107 (15)	205 (117)
		Pb	93 (8)	118 (11)	237 (23)	355 (149)	205 (117)
Violet 3R	25	-	6 (1)	8 (6)	10 (4)	20 (7)	48 (3)
	2,500	-	4 (1)	18 (6)	8 (4)	22[c]	49 (4)
	25	Ar	8 (2)	8 (1)	19 (5)	48 (4)	96 (7)
	50		8 (1)	7 (3)	23 (3)	40 (4)	88 (24)
	250		7 (1)	5 (2)	18 (5)	44 (8)	98 (12)
	500		8 (3)	10 (7)	16 (6)	51 (3)	91 (15)
	2,500		8 (3)	9 (6)	16 (5)	54 (15)	107 (32)
	5,000		7 (4)	9 (5)	16 (3)	38 (8)	78 (19)
	25	Pb	8 (4)	13 (8)	11 (2)	28 (10)	65 (11)
	50		10 (5)	7 (7)	10 (4)	34 (14)	65 (18)
	250		9 (4)	9 (5)	8 (1)	37 (16)	68 (16)
	500		8 (1)	8 (4)	9 (3)	34 (9)	58 (11)
	2,500		8 (4)	6 (3)	8 (3)	34 (15)	73 (19)
	5,000		7 (2)	7 (3)	11 (3)	30 (9)	71 (19)

a. - = No activation; Ar = Aroclor; Pb = Phenobarbital.
b. Mean of three replicates (standard deviation).
c. Toxic to bacteria.

TABLE A-20. MACROLEX VIOLET 3R IN VITRO ASSAY WITH SALMONELLA TYPHIMURIUM PREINCUBATION ASSAY
ORNL

Compound	µg/Plate	Activation[a]	Histidine Revertants Per Plate by Strain TA 1535	TA 1538	TA 98	TA 100
DMSO	100	-	9[b] (1)	9 (6)	32 (10)	112 (37)
		Ar	12 (4)	18 (6)	35 (10)	130 (34)
		Pb	14 (5)	20 (15)	32 (5)	117 (17)
Positive Control		-	91 (10)	237 (23)	355 (149)	205 (117)
		Ar	91 (10)	237 (23)	107 (15)	205 (117)
		Pb	91 (10)	237 (23)	107 (15)	205 (117)
Violet 3R	250	-	11 (6)	7 (4)	25 (8)	98 (31)
	2,500	-	13 (3)	7 (3)	30 (10)	119 (28)
	25	Ar	9 (2)	11 (3)	36 (3)	120 (25)
	50		7 (2)	11 (3)	29 (8)	92 (14)
	250		9 (2)	9 (3)	32 (7)	92 (8)
	500		8 (6)	17 (2)	31 (8)	87 (30)
	2,500		8 (2)	9 (4)	39 (8)	87 (30)
	5,000		8 (3)	11 (5)	35 (14)	72 (13)
	25	Pb	8 (3)	6 (3)	17 (13)	98 (37)
	50		9 (3)	11 (5)	20 (11)	95 (22)
	250		10 (5)	7 (4)	18 (11)	94 (17)
	500		6 (3)	6 (3)	16 (11)	85 (19)
	2,500		8 (2)	10 (6)	22 (14)	114 (36)
	5,000		6 (3)	10 (3)	19[c] (15)	83 (21)

a. - = No activation; Ar = Aroclor; Pb = Phenobarbital.
b. Mean of two to three replicates (standard deviation).
c. Toxic to bacteria.

APPENDIX B

MUTAGENIC INDEX

Tables

Mutagenic Index: Average plate counts divided by average spontaneous count for the same bacterial strain.

Underlined values are greater than a twofold increase over spontaneous levels. Data are from standard plate incorporation assay.

TABLE B-1. MACROLEX RED 1069 MUTAGENIC INDEX
USEPA

Act.	µg/Plate	TA 1535	TA 1537	TA 1538	TA98				TA100		
None	0.1					1.1	1.0		1.1	1.0	
	0.5					0.9	1.0		1.2	1.3	
	1					1.1	1.2		1.2	1.2	
	5					1.1	1.0		1.3	1.2	
	10				1.0	0.8	1.5		1.3	1.1	
	50	1.2	3.3	1.3	1.1	0.9	2.0	0.8	1.3	1.4	1.0
	100	1.5	2.3	2.2	1.0	1.1	0.7	0.8	1.3	1.1	0.9
	500	1.2	3.7	2.3	1.0	1.3	1.3	1.1	1.0	0.9	1.1
	1,000	1.6	4.1	3.3	1.0	1.2	1.3	1.8	0.9	0.8	1.1
	2,000	1.2	4.0	6.5				2.1		1.1	
	3,000	0.6	2.4	6.7				2.1		1.0	
	5,000				1.1	1.8	2.6		1.0		
Aroclor	0.1					1.2	1.0		1.0	1.4	
	0.5					1.0	1.3		1.1	1.7	
	1					1.0	1.3		1.1	1.5	
	5					1.3	1.3		1.2	1.7	
	10				1.1	1.8	1.3		1.3	1.7	
	50	1.4	4.0	1.8	1.1	2.2	1.6	1.5	1.1	1.6	1.0
	100	1.0	3.9	2.5	0.8	3.2	2.1	2.3	1.0	1.3	1.1
	500	1.1	4.3	7.0	1.0	5.3	6.4	4.8	1.0	1.3	1.2
	1,000	1.1	8.1	12.1	0.9	7.6	8.2	8.1	0.9	1.1	1.2
	1,200			34.3							
	2,000	1.7	16.1	27.5				17.1		1.4	
	3,000	1.6	11.4					14.7		1.4	
	5,000				1.3	18.8	20.3		1.3	1.5	

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TABLE B-2. MACROLEX RED 1069 MUTAGENIC INDEX
ORNL

Act.	µg/Plate	TA 1535	TA 1537		TA 1538		TA 98		TA 100
Aroclor	1.25	1.0							
	2.5	1.1							
	5	1.1							
	25	0.9	1.6	1.0	2.1	1.9	1.7		1.3
	50	1.0	2.3	1.7	3.1	2.9	2.0		1.0
	125		3.5	2.5	4.9	4.1			
	250	1.0	4.6	3.6	9.1	5.9	4.4		1.2
	500	1.0	6.2	5.0	14.6	10.9	7.5		1.5
	750		6.4	5.1	15.9	13.7	7.9		
	1,000		6.6	4.1	15.7	11.3	8.1		
	1,250		14.1	9.8	20.2	27.3	14.8		
	2,500	1.1	21.5	15.3	49.7	38.9	21.2	13.9	1.8
	5,000	1.0	33.8	22.7	76.9	58.7	31.8	22.2	1.5
	7,500						31.5		
	10,000		35.3	21.6			32.3		
Pheno-barbital	25		0.9		2.1		1.0		1.5
	50		1.4		2.4		1.0		1.3
	250		2.0		2.4		1.1		1.4
	500		1.9		4.5		1.4		1.3
	2,500		2.6		5.3		1.8		1.3
	5,000		2.7		9.8		2.5		1.2
	7,500				10.8		3.1		
	10,000				11.9		3.1		

TABLE B-3. AMOPLAST RED PC (RED 10618) MUTAGENIC INDEX
USEPA

Act.	µg/Plate	TA 1535	TA 1537	TA 1538	TA 98			TA 100			
None	10	1.1	1.6	4.1			5.0				1.9
	30	1.1	2.3	8.6			9.6				2.2
	50	1.4	2.5	8.6	11.3	12.5	11.8		2.2	2.7	2.3
	100	0.8	3.0	16.4	19.2	16.2	16.8		2.7	3.4	2.6
	150				28.5	21.1			2.5	3.9	
	200				25.5	21.7			3.4	4.5	
	250				34.9	95.6		3.5	3.0	3.4	
	300	1.2	3.4	34.0			25.7				2.3
	500	0.8	2.5	35.6	37.2	23.9	30.0	3.9	3.9	4.8	2.9
	750							4.2			
	1,000							5.6			
	1,500							5.4			
	2,500							4.9			
	3,500							3.6			
Aroclor	10	1.5	1.8	4.8			10.5				2.2
	30	1.4	4.4	10.0			35.1				2.9
	50	1.5	6.3	19.3	54.7	59.5	52.9		3.5	5.0	3.8
	100	1.7	10.5	44.2	90.3	89.1	66.6		5.6	7.3	6.1
	120		12.3								
	150				84.1	82.8			6.9	9.1	
	200		10.8		121.4	81.2			8.5	10.1	
	250				86.9	80.4		7.6	7.6	8.8	
	300	1.3		62.4			93.1				8.2
	500	1.5		66.9	131.1	113.6	89.5	4.5	9.9	9.9	8.4
	750							2.5			
	1,000							1.7			
	1,500							1.3			
	2,500							0.7			
	3,500							0.3			

TABLE B-4. AMOPLAST RED PC (RED 10618) MUTAGENIC INDEX
ORNL

	Without Activation					Aroclor Activation				
µg/Plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100	TA 1535	TA 1537	TA 1538	TA 98	TA 100
12.5							1.4	1.5	1.4	
25			1.8	1.3		0.9	1.7	1.9	1.3	1.2
50			3.5	1.1		1.4	2.2	3.5	1.8	1.1
75								4.2	1.7	
100								3.7	2.0	
125			3.1				4.2	5.4	2.7	
250	0.3	1.1	4.4	1.1	0.3	1.1	4.2	9.3	4.1	1.3
500		1.2	7.4	1.5		1.8	4.2	12.7	5.9	1.5
750		1.3	7.9	2.0			4.2	13.9	6.5	
1,000		0.9	7.9	1.5			4.5	15.9	7.9	
1,250		2.8	14.0	2.7			8.2	24.1	10.1	
2,500	0.4	3.5	22.2	3.1	0.1	1.6	8.9	29.7	15.0	1.5
5,000		3.4	31.4	3.9		1.4	9.3	30.1	15.3	2.1
7,500		2.9								
10,000		2.8								
			Phenobarbital Activation							
12.5		2.4		1.3						
25	1.0	2.8	3.0	1.6	1.1					
50	0.9	3.0	4.4	1.9	1.1					
125		3.5	5.9	2.1						
250	1.1	4.2	8.6	2.6	1.2					
500	1.0	3.5	10.6	3.5	1.2					
750			11.6	4.4						
1,000			12.6	4.4						
1,250		8.5	22.9	6.2						
2,500	0.9	10.0	31.6	9.4	1.3					
5,000	0.9	7.9	38.4	10.7	1.4					

TABLE B-5. OIL RED G MUTAGENIC INDEX
USEPA

Act.	µg/Plate	TA 1535		TA 1537		TA 1538		TA 98				TA 100					
None	5							1.6	0.9				0.9				
	10	0.8	1.1	1.4	1.3	1.0	0.6			1.3	0.8			0.9	0.8		
	30	0.9	1.1	1.8	0.8	1.3	0.6			1.1	0.7			0.7	0.8		
	50	0.9	1.1	2.4	1.0	0.8	0.7	1.1	0.5	1.1	0.9		0.8	1.0	0.8	0.9	
	62.5											1.2					
	100	1.1	1.2	1.6	0.9	1.0	0.6	1.2	0.8	1.1	0.9		1.1	0.9	1.2	0.9	1.0
	125											1.0					
	250											1.2					
	300	0.9	1.2	1.8	1.2	1.4	0.9	1.0	1.0	1.1	0.9		0.9		0.9	0.9	0.9
	500	0.7	1.5	1.8	0.9	1.2	0.7	1.5	0.9	1.6	1.1	1.4	1.1	0.8	1.1	1.0	0.6
	1,000											1.5	1.0				
	1,500											1.4					
	5,000												1.2				
Aroclor	5							1.5	1.1				1.2		2.0		
	10	0.7	0.7	1.3	1.2	0.8	0.7			0.9	0.9				1.7	1.5	
	30	0.9	0.8	1.0	1.0	0.6	0.7			0.8	0.9				1.4	1.4	
	50	0.6	0.8	2.5	1.3	0.8	0.8	1.2	1.0	1.1	0.9		1.1		1.8	1.4	1.4
	62.5											2.0					
	100	0.6	0.8	2.3	1.4	0.7	0.8	0.9	1.2	1.1	0.9		2.0	1.1	2.1	1.3	1.7
	125											1.7					
	250											1.6					
	300	0.6	0.6	1.3	1.2	0.8	0.7	1.3	1.2	0.9	0.9		1.1		1.8	1.5	1.4
	500	0.6	0.9	1.1	1.3	1.0	1.0	1.4	1.2	0.8	1.0	1.4	1.5	1.3	1.7	1.4	1.4
	1,000											1.7	1.5				
	1,500											1.6					
	5,000												1.2				

TABLE B-6. OIL RED G MUTAGENIC INDEX
ORNL

	Aroclor Activation					Phenobarbital Inactivation				
µg/Plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100	TA 1535	TA 1537	TA 1538	TA 98	TA 100
25	0.9	1.0	0.9	1.1	1.1	0.9	1.5	0.8	0.8	1.0
50	1.0	1.8	0.9	1.0	1.0	0.7	1.0	0.8	0.5	1.0
250	1.1	1.2	0.7	0.9	1.1	1.7	1.0	0.8	0.8	0.7
500	0.7	1.2	0.7	1.0	0.9	0.9	1.5	0.6	0.9	0.7
2,500	0.8	1.1	1.1	0.9	1.0	1.1	1.3	1.1	0.7	1.1
5,000	0.6	1.3	1.1	1.0	1.1	0.9	1.3	1.0	0.9	0.8
	Without Activation									
250	2.0	-	0.5	0.8	1.0					
2,500	1.4	-	1.0	0.5	0.6					

TABLE B-7. RESIREN VIOLET TR MUTAGENIC INDEX
ORNL

Activation	µg/Plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
Aroclor	1.25			1.7	1.2	
	2.5			3.3	1.1	
	5			4.2	2.1	
	12.5		2.9	5.5	3.2	
	25	1.4	4.6	8.5	4.1	0.9
	50	1.1	6.2	11.1	4.8	0.8
	125		6.4	9.5	5.0	
	250	0.9	5.6	11.5	5.0	1.0
	500	1.1	6.3	12.5	5.6	0.9
	750		5.6			
	1,000		5.0			
	1,250		4.9		4.1	
	2,500	0.6	3.6	5.1	3.4	0.5
	5,000	0.4	2.1	2.1	1.6	0.1
Phenobarbital	1.25			6.6	2.2	
	2.5			9.9	4.0	
	5			14.4	4.4	
	12.5		7.9	19.5	5.2	
	25	0.8	9.0	21.4	5.2	1.1
	50	1.0	10.0	17.9	5.5	1.2
	125		10.5	19.8	6.1	
	250	1.1	9.5	20.5	5.8	1.3
	500	0.7	8.3	19.9	5.3	1.1
	1,250		8.0		3.5	
	2,500	0.7	5.0	9.1	2.4	0.6
	5,000	0.5	2.2	5.6	1.1	0.4

TABLE B-8. MACROLEX VIOLET B MUTAGENIC INDEX
ORNL

Activation	µg/Plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
None	12.5		2.2			
	25	0.9	2.8			
	50		3.3			
	125		3.9			
	250	1.0	5.2	1.3	1.3	0.9
	500		4.1			
	1,250		4.2			
	2,500	0.9	6.3	1.4	1.1	0.9
	5,000		6.3			
Aroclor	25	1.1	0.9	1.4	1.2	1.0
	50	1.2	0.9	1.4	1.3	0.7
	250	1.1	1.1	1.7	1.4	0.9
	500	1.0	0.9	1.9	1.2	1.0
	2,500	1.1	1.8	1.9	1.4	0.9
	5,000	0.9	1.7	2.1	1.4	0.8
Phenobarbital	25	0.6	1.0	1.0	0.9	0.9
	50	0.9	0.6	0.9	1.0	1.1
	250	0.9	0.9	0.9	0.9	1.1
	500	1.3	0.9	1.0	0.9	1.1
	2,500	1.1	1.4	1.5	1.0	1.1
	5,000	0.8	1.6	1.2	1.0	1.0

TABLE B-9. MACROLEX VIOLET 3R MUTAGENIC INDEX
ORNL

Activation	µg/Plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
Aroclor	25	1.1	0.6	1.3	1.4	1.1
	50	1.0	0.5	1.5	1.1	1.0
	250	0.9	0.4	1.2	1.3	1.0
	500	1.0	0.8	1.1	1.5	1.0
	2,500	1.1	0.7	1.1	1.5	1.2
	5,000	0.9	0.7	1.1	1.1	0.9
Phenobarbital	25	0.8	1.3	1.4	0.7	0.9
	50	1.0	1.0	1.3	0.9	0.9
	250	0.9	0.9	1.0	1.1	1.0
	500	0.8	0.8	1.1	0.9	0.8
	2,500	0.9	0.4	1.0	0.9	1.0
	5,000	0.7	0.7	1.4	0.8	1.0

APPENDIX C

SUMMARY OF STATISTICAL ANALYSES, REGRESSION SLOPES

Tables

Underlined values fit the mutagenicity and Poisson parameters of the nonlinear Poisson model and are a positive mutagenic response.

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TABLE C-1. MACROLEX RED 1069 SUMMARY OF STATISTICAL ANALYSES, REGRESSION SLOPES

Strain	Without Activation		Aroclor Activation		Phenobarbital Activation	
	Linear	Nonlinear	Linear	Nonlinear	Linear	Nonlinear
TA1535	0.005 (-0.001, 0.010)[a]	0.023 (0.007, 0.030)	0.002 (-0.000, 0.004) 0.154 (-1.223, 1.531)	0.001 (0.000, 0.006) 0.002 (0.000, -----)		
TA1537	0.025 (0.007, 0.044)	0.050 (0.034, 0.076)	0.047 (0.040, 0.054) 49.889 (43.021, 56.756)	0.037 (0.034, 0.040) 153.278 (147.861, 158.895)	0.008 (0.005, 0.012)	0.014 (0.010, 0.022)
TA1538	0.015 (0.013, 0.017)	0.013 (0.011, 0.016)	0.238 (0.174, 0.303) 0.216 (0.200, 0.232)	2.246 (1.842, 2.740) 0.201 (0.200, 0.201)	11.760 (8.674, 14.846)	11.565 (10.750, 12.440)
TA98	0.105 (-0.196, 0.405) 0.003 (0.002, 0.004) 0.004 (0.003, 0.006) 0.014 (0.011, 0.016)	0.988 (0.000, ----) 0.003 (0.000, 111.955) 0.004 (0.000, 0.055) 0.101 (0.008, 1.202)	10.178 (2.342, 18.014) 0.089 (0.079, 0.099) 0.100 (0.090, 0.110) 0.212 (0.201, 0.224) 138.044 (121.473, 154.616)	9.858 (8.573, 11.335) 0.087 (0.073, 0.105) 0.187 (0.112, 0.312) 0.800 (0.661, 0.969) 254.122 (249.816, 258.502)	10.636 (8.590, 12.683)	16.254 (10.705, 24.680)
TA100	-0.003 (-0.007, 0.000) -0.003 (-0.008, 0.003) 0.016 (-0.015, 0.048)	0.004 (0.001, 0.021) 0.003 (0.001, 0.009) 0.048	0.003 (-0.000, 0.007) -0.000 (-0.007, 0.006) 0.015 (0.008, 0.021) 0.023 (0.012, 0.034)	0.003 (0.000, 0.130) 0.009 (0.004, 0.020) 0.038 (0.001, 1.149) 0.089 (0.066, 0.120)	0.036 (-0.050, 0.122)	0.067 (0.026, 0.169)

a. Ninety-five percent confidence limits.

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TABLE C-2. AMOPLAST RED PC (RED 10618) SUMMARY OF STATISTICAL ANALYSES, REGRESSION SLOPES

Strain	Without Activation		Aroclor Activation		Phenobarbital Activation	
	Linear	Nonlinear	Linear	Nonlinear	Linear	Nonlinear
TA1535	0.003 (-0.020, 0.025)[a]	0.012 (0.000, 2.161)	0.002 (-0.008, 0.012) 0.012 (0.000, 0.023)	0.012 (0.005, 0.026) 0.013 (0.005, 0.033)	0.004 (-0.013, 0.022)	0.000
TA1537	0.239 (0.162, 0.317) 10.036 (7.304, 12.768)	0.396 (0.183, 0.856) 57.606 (47.439, 69.952)	1.502 (1.387, 1.616) 0.051 (0.036, 0.067)	5.118 (3.545, 7.390) 0.061 (0.058, 0.064)	0.047 (0.037, 0.057)	0.061 (0.057, 0.066)
TA1538	1.040 (0.870, 1.210) 0.050 (0.045, 0.055)	1.591 (1.384, 1.829) 0.050 (0.049, 0.051)	3.451 (2.715, 4.186) 0.177 (0.156, 0.199)	10.377 (9.948, 10.824) 0.264 (0.258, 0.271)	0.060 (0.053, 0.068)	0.063 (0.061, 0.064)
TA98	1.209 (0.779, 1.640) 5.489 (2.841, 8.137) 1.255 (1.021, 1.489) 0.038 (0.020, 0.055)	3.972 (3.037, 5.195) 15.334 2.138 (1.913, 2.388) 0.052 (0.048, 0.057)	4.797 (2.655, 6.939) 4.080 (2.084, 6.077) 9.231 (6.708, 11.753) 0.025	7.771 (7.556, 7.993) 5.283 (5.247, 5.319) 25.385 (25.029, 25.747) 0.168	0.079 (0.069, 0.089)	0.154 (0.140, 0.168)
TA100	0.456 (0.333, 0.579) 0.443 (0.280, 0.606) 0.547 (0.294, 0.801) 0.196 (0.077, 0.315)	0.813 (0.749, 0.883) 0.524 (0.158, 1.735) 0.895 (0.622, 1.287) 0.325 (0.308, 0.343)	0.067 (-0.810, 0.944) 1.436 (0.930, 1.941) 1.270 (0.553, 1.987) 2.274 (1.806, 2.742) 0.078 (-0.053, 0.209)	3.869 (3.155, 4.744) 4.525 (3.870, 5.291) 4.667 (4.253, 5.122) 0.102 (0.071, 0.145)	0.042 (-0.019, 0.104)	0.049 (0.017, 0.142)

a. Ninety-five percent confidence limits.

TABLE C-3. OIL RED G SUMMARY OF STATISTICAL ANALYSES, REGRESSION SLOPES

Compound	Without Activation		Aroclor Activation		Phenobarbital Activation	
	Linear	Nonlinear	Linear	Nonlinear	Linear	Nonlinear
TA1535	0.019	0.133	-0.084	0.005	-0.008	0.000
	(-0.019, 0.056)[a]	(0.001, 21.844)	(-0.169, 0.001)	(0.000, 13.440)	(-0.037, 0.020)	(0.000, 0.000)
	0.037	0.048	-0.001	0.002		
	(-0.084, 0.158)	(0.002, 1.158)	(-0.008, 0.006)			
	-0.000	0.000	0.007	0.013		
	(-0.003, 0.003)		(-0.023, 0.036)	(0.005, 0.033)		
TA1537	0.004	0.027	0.207	0.245	-0.003	0.000
	(-0.011, 0.020)	(0.004, 0.163)	(0.101, 0.313)	(0.120, 0.501)	(-0.016, 0.010)	
	-0.034	0.036	0.036	0.039		
	(-0.115, 0.046)	(0.001, 1.059)	(-0.044, 0.115)	(0.000, ----)		
			0.001	0.001		
			(-0.001, 0.003)	(0.001, 0.004)		
TA1538	0.011	0.007	-0.098	0.011	0.000	0.000
	(-0.003, 0.025)	(0.001, 0.083)	(-0.232, 0.035)	(0.000, 0.261)	(-0.000, 0.001)	(0.000, 0.005)
	0.007	0.000	0.008	0.000		
	(-0.009, 0.022)		(-0.003, 0.018)			
			0.002	0.001		
			(-0.001, 0.004)			
TA98	0.004	0.018	0.004	0.036	-0.021	0.018
	(-0.017, 0.024)		(-0.050, 0.057)	(0.003, 0.477)	(-0.080, 0.038)	(0.008, 0.038)
	0.005	0.000	0.032	0.035		
	(-0.027, 0.037)		(-0.023, 0.088)	(0.000, 101.609)		
	0.015	0.017	0.053	0.164		
	(0.004, 0.027)	(0.010, 0.030)	(-0.091, 0.197)	(0.046, 0.585)		
	0.009	0.009	-0.001	0.057		
	(-0.001, 0.018)	(0.001, 0.152)	(-0.010, 0.008)	(0.000, 1057.24)		
			0.044	0.055		
			(-0.025, 0.113)	(0.042, 0.071)		
TA100	0.029	0.033	0.064	0.164	0.047	0.108
	(0.001, 0.056)	(0.008, 0.134)	(-0.037, 0.166)		(-0.071, 0.166)	(0.063, 0.185)
	-0.081	0.631	0.474	0.526		
	(-0.587, 0.424)	(0.481, 0.826)	0.136, 0.812)	(0.405, 0.683)		
	0.053	0.130	0.011	0.078		
	(0.028, 0.078)	(0.028, 0.608)	(-0.041, 0.063)	(0.025, 0.240)		
	0.336	0.513	6.549	6.570		
	(0.008, 0.664)	(0.245, 1.073)	(0.954, 12.144)	(1.617, 26.695)		
	-0.129	0.000	0.041	0.045		
	(-0.335, 0.077)		(-0.000, 0.083)	(0.011, 0.188)		
	-0.012	0.017	0.625	1.023		
	(-0.062, 0.039)	(0.000, 6.604)	(-0.105, 1.355)	(0.688, 1.522)		
			0.039	0.146		
			(-0.090, 0.168)	(0.102, 0.208)		

a. Ninety-five percent confidence limits.

TABLE C-4. VIOLET DYES SUMMARY OF STATISTICAL ANALYSES, LINEAR REGRESSION SLOPES

Compound	Act.	TA1535	TA1537	TA1538	TA98	TA100
Resiren Violet TR	Ar	-0.0101	1.391 (0.954, 1.828)[a]	2.848 (2.275, 3.422)	5.308 (4.160, 6.455)	0.0240
	Pb	0.009 (-0.019,0.036)	0.505 (0.233, 0.777)	5.820 (4.265, 7.374)	11.644 (7.132, 16.156)	0.059
Macrolex Violet B	—	-0.000 (-0.002, 0.002)	0.166 (0.101, 0.231)			
	Ar	0.000 (-0.026, 0.026)	0.005 (0.003, 0.006)	0.030 (0.007, 0.053)	0.003 (-0.003, 0.009)	-0.016 (-0.096, 0.064)
	Pb	0.003 (-0.031, 0.037)	0.002 (0.001, 0.004)	0.002 (0.001, 0.003)	-0.009 (-0.078, 0.061)	0.027 (-0.032, 0.085)
Macrolex Violet 3R	Ar	0.001 (-0.000, 0.001)	-0.001 (-0.013, 0.011)	0.004 (-0.027, 0.034)	0.004 (0.000, 0.008)	0.007 (-0.002, 0.016)
	Pb	0.000 (-0.002, 0.002)	-0.001 (-0.002, 0.000)	-0.004 (-0.019, 0.012)	0.005 (-0.060,0.069)	0.003 (-0.004, 0.010)

a. Ninety-five percent confidence limits.

TABLE C-5. VIOLET DYES SUMMARY OF STATISTICAL ANALYSES, NONLINEAR REGRESSION SLOPES

Compound	Act.	TA1535	TA1537	TA1538	TA98	TA100
Resiren Violet TR	Ar	0.0077 (0.0002, 0.312)[a]	1.185	2.344 (2.292, 2.397)	4.271 (4.123, 4.424)	0.000
	Pb	0.001 (0.000,0.057)	0.725 (0.682, 0.770)	5.051 (4.931, 5.175)	11.169 (10.776, 11.576)	0.038 (0.009, 0.155)
Macrolex Violet B	--	0.000	0.144 (0.137, 0.151)			
	Ar	0.008 (0.001, 0.066)	0.019 (0.003, 0.121)	0.036 (0.022, 0.057)	0.005 (0.004, 0.006)	0.015 (0.005, 0.043)
	Pb	0.010 (0.003, 0.032)	0.008 (0.000, 0.221)	0.016 (0.003, 0.106)	0.000 (0.012, 0.085)	0.032
Macrolex Violet 3R	Ar	0.000 (0.000, 52.951)	0.002 (0.001, 0.008)	0.025 (0.015, 0.043)	0.040 (0.015, 0.104)	0.096 (0.060, 0.154)
	Pb	0.000 (0.000, 8.860)	0.001 (0.000, 0.002)	0.006 (0.001, 0.048)	0.006 (0.002, 0.019)	0.059 (0.026, 0.136)

a. Ninety-five percent confidence limits.

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